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A Rapid Modified Antibody-Capture Enzyme-Linked Immunosorbent Assay (ELISA) for Detection HSV-1 Primary Infection in Human.

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Abstract:

An antibody-capture enzyme-linked immunosorbent assay (ELISA) based on the reaction between anti human IgM and serum IgM and HSV-1 conjugated with horseradish peroxidase (HRP) was developed. The specificity and sensitivity of the developed HSV-1 IgM ELISA were evaluated and compared with a commercially available test kit by using sets of well defined sera of known HSV-1 primary infection. For validation, 526 serum samples have been tested. The HSV-1 IgM ELISA had acceptable sensitivity and specificity, which were comparable with that of the commercial test kit. Both assays showed some cross-reactivity with sera containing anti-HSV-2 antibodies.

Key Words: Herpes Simplex Virus, ELISA, IgM.

Introduction:

Herpes simplex virus type 1 (HSV-1) is an ubiquitous pathogen of humans that usually causes either asymptomatic infection or skin and mucosal diseases. HSV-1 causes different clinical symptoms in about 10% of primary infections. The major clinical manifestations associated with HSV-1 infections are gingivostomatitis, keratitis, conjunctivitis, vesicular eruptions of the skin, encephalitis, eczema, and lethal infections of newborns (Prober et al, 1987; Roizman et al,1996; Whitley, 1996).

In recent years because of the increasing number of immunocompromised patients, there has been great interest in simple and sensitive methods to determine both acute and past infections with HSV-1 (Cann et al, 1997; Desselberger, 1995).

IgM antibody against HSV-1 has been detected early in infection in sera and cerebrospinal fluid with a variety of immunoassay methods (Doerr et al, 1987; Linde et al, 1997; Sharief et al, 1999).

Comparison between different assay methods with respect to specificity, and sensitivity shows ELISA to be the most suitable of the available techniques (Johnston et al, 1992; Linde et al, 1997; Sharief et al, 1999).

Materials and Methods:

Virus and antigen preparation: HSV-1 (Strain MacIntyre) was propagated in HeLa cell cultures. After appearance of complete cytopathic effect (CPE), the supernatant fluid was collected, and centrifuged at 2500X g for 15 min to remove the cellular debris. Polyethyleneglycol 6000 (Sigma chemicals, Dorset, UK) was added to the supernatant to a final concentration of 5%. The mixture was then incubated for 6hr at 4°C, and centrifuged at 12000X g at 4°C for 20 min. The resulting pellet was resuspended in Tris-HCl at pH 7.4.

Control antigen was prepared from uninfected HeLa cells by sonication in Tris-HCl for 8 min, and centrifugation at 2500X g for 15 min to remove cellular debris.

Conjugation of HSV-1 with horseradish peroxidase: HSV-1 was conjugated to horseradish peroxidase (HRP)(Sigma-Aldrich, Dorset, UK) by the periodate method (Kurstak, 1985). HRP was dissolved in 0.1M $NaHCO_3$ at the concentration of 10 mg/ml, 0.05M $NaIO_4$ and activated by mixing for 3hr at room temperature in darkness. HSV-1 was then added to the activated HRP. The conjugate was eluted in 2ml of 0.1M carbonate buffer with pH 9.2.

Sera: Using both in-house and commercial ELISA method, HSV-1 IgM antibody was measured in 500 serum samples collected from normal persons. Sera were diluted 1:250 in phosphate buffer saline pH 7.4 with 0.05% Tween-20 (PBST). More serum samples were also collected from a further 26 patients with primary HSV-1 infection.

HSV-1 IgM ELISA procedure: A microtiter ELISA plate (Greiner Max abs. Labortechnik, Germany) was coated with 100µl of 1:500 anti human IgM antibody (Sigma-Aldrich, Dorset, UK) diluted in 0.1M carbonate buffer pH 9.6, and incubated in 4°C overnight. The plate was then washed five times with PBST prepared in deionized water. Following blocking by PBS with 0.5% Tween-20, at room temperature for 60 min, the plate was washed , and human serum at the dilution of 1:250 was added to the plate and incubated for 60 min at room temperature.

Following washing, the plate was incubated in a 1:500 dilution of conjugated HSV-1-peroxidase at 37°C for 45min. OPD (Sigma-Aldrich, Dorset, UK) was added after washing, and incubated in darkness at 37°C for 30min. The reaction was stopped using 50µl of 2M H_2SO_4. Color development was measured at 492nm with microplate reader (Labsystems, UK).

Evaluation of the ELISA results: The HSV-1 IgM ELISA results were compared with a commercially available kit (IBL, Hamburg, Germany). The inhibition percentage of each serum sample tested by the HSV-1 IgM ELISA was calculated according to the following formula:

%Inhibition = 100% - OD of tested sample x 100/mean OD of negative controls

The specificity of the ELISA was expressed as the percentage of serum samples obtained from uninfected persons, which gave unambiguously negative results at the certain cut-off value. The sensitivity of the ELISA was expressed as the percentage of the positive serum samples obtained from

primary infected persons with HSV-1, which gave unambiguously positive results at the certain cut-off value. For the HSV-1 IgM ELISA, the cut-off value of 45% was chosen as the best for the highest specificity and sensitivity (Table 1). For the commercial test the cut-off value was used according to the manufacturers' procedures.

Results:

Optimization of the HSV-1 IgM ELISA conditions: Appropriate conditions for the HSV-1 IgM ELISA were determined by a series of checkerboard ELISA, to obtain the optimum concentration of antigen, serum samples, and anti human IgM Abs, and appropriate pH and type of buffers, and blocking reagents.

Determination of the positive/negative cut-off value: The inhibition percentage of the test ranged from –15 to 100% and over this entire rang cut-off values were determined at intervals of 5%. Each of those cut-off values was used as a limit that classified the test signal as either positive or negative. At each limit the sensitivity and specificity of the HSV-1 IgM ELISA were calculated (Table.1).

The mean blocking percentage of negative sera was 35. The mean blocking plus 2 standard deviations was therefore 44% (9 + 35 = 44%). Based on these results the optimal cut-off value for the HSV-1 IgM ELISA was selected 45%.

Validation of the HSV-1 IgM ELISA and comparison with the commercial ELISA: The specificity and sensitivity of the HSV-1 ELISA were determined and compared with a commercially available test kit using a set of well-defined sera. Samples were tested according to the procedure of HSV-1 ELISA as described in section 2 and the manufactures' instructions.

Twenty-eight of the 500 samples gave a false positive reaction, giving a specificity of 94.40%. Thirty-two out of 526 sera gave false negative results, giving a sensitivity of 93.91% (Table.2). The possibility of presence of cross-reaction was examined with sera containing antibodies against HSV-2. All sera were negative by the HSV-1 test.

Table 1. Sensitivity and Specificity of the HSV-1 IgM ELISA at cut-off values with intervals of 5% over the entire range of observed inhibition percentages.

Cut –off value (% blocking)	Sensitivity (%)	Specificity (%)
-15	100	0
-10	100	0
-5	100	0.76
0	100	3.61
5	100	9.69
10	100	21.10
15	100	41.44
20	100	56.27
25	100	67.11
30	98.60	87.07
35	97.20	90.11
40	95.20	92.96
45	94.40	93.91
50	90.40	96.38
55	85.00	97.14
60	79.60	98.09
65	75.60	98.66
70	69.40	99.04
75	62.80	100
80	52.60	100
85	45.40	100
90	33.60	100
95	11.20	100
100	0	100

Table 2. Specificity and Sensitivity for HSV-1 IgM ELISA in comparison with a commercial tests at certain cut-off values [a].

Assay	Specificity (%)[b]	Sensitivity (%)[c]
HSV-1 IgM ELISA	94.40 (472/500)	93.91 (494/526)
Commercial Test	93.80 (469/500)	92.39 (486/526)

[a] For the HSV-1 IgM ELISA cut-off values of 45% was chosen, for the commercial test cut-off value was used in accordance with manufacturer's procedure.
[b] Numbers in parentheses are the number of negative test results to the numbers of tested negative sera.
[c] Numbers in parentheses are the numbers of positive test results to the number of tested positive sera.

Discussion:

The detection of HSV-1 IgM provides a powerful, rapid and economic method for the diagnosis of HSV-1 primary infection. Rapid diagnosis of HSV-1 is of particular importance for prevention of nosocomial spread of HSV-1 among immunocompromised patients; in differentiation of HSV-1 and VZV infection in eczema herpeticum. In addition, identification of pregnant woman at risk of transmitting HSV-1 to newborns is an increasingly important area as suitable antiviral therapy can be initiated (Whitley et al, 1996). Finally, HSV-1 IgM detection provides a rapid and sensitive method for diagnosis of HSV-1 infection of the CNS and in the a etiological diagnosis of acute aseptic meningitis without the isolation of the virus.

A number of tests can differentiate between HSV-1 and HSV-2 antibodies. The definitive and most reliable of these is western blotting against intact gG1 and gG2 proteins (WHO Workshop, 1995). However, the procedure is time consuming, relatively expensive and not undertaken frequently outside specialist laboratories. Enzyme immunoassays (EIAs) are both faster and simpler and more readily applied in routine laboratories (Ashley et al, 1991).

In the present study, HSV-1 IgM ELISA was investigated to determine its ability in the detection of IgM antibody. Although only a limited number of serum samples were available for study, there seems that HSV-1 ELISA has acceptable sensitivity and specificity. Further development of these assays is desirable if they are to be applied more widely. In addition both the sensitivity and specificity of testing must be improved. As for the differentiation of herpes simplex virus type 1 and 2 the use of glycoprotein antigens produced by recombinant technology may ultimately be the key to the production of more sensitive and specific immunoassay techniques (Eis-Hubinger et al. 1999).

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